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Selective and Differential Medium for Recovery of *Pseudomonas cepacia* from the Respiratory Tracts of Patients with Cystic Fibrosis

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A selective and differential medium, OFPBL (oxidation-fermentation base supplemented with agar, lactose, and two antimicrobial agents), for the isolation of *Pseudomonas cepacia* from respiratory specimens of patients with cystic fibrosis was developed and tested. Among 725 specimens submitted from seven centers over a 4- to 6-month period, 58 (8%) yielded *P. cepacia* on OFPBL; only 19 of these were recovered on MacConkey or sheep blood agar ($P < 0.001$). No isolate was recovered on MacConkey or sheep blood agar alone. Ranges of recovery rates among centers were 0 to 15% on OFPBL and 0 to 10% on MacConkey or sheep blood agar. Ninety percent of *P. cepacia* isolates were detected on OFPBL in ≤3 days. Other nonfermenters and yeasts isolated on OFPBL were distinguished from *P. cepacia* by failure to acidify the medium. The new medium was clearly superior to MacConkey and sheep blood agars for the isolation of *P. cepacia* from the respiratory secretions of patients with cystic fibrosis.

Patients with cystic fibrosis (CF) are predisposed to colonization and acute and chronic pulmonary infections caused by microorganisms such as *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Pseudomonas cepacia* (2, 6, 7, 12). It has been hypothesized that lytic enzymes of these microbes or of inflammatory cell origin contribute to progressive destruction of lung tissue (1, 2, 6, 9) and, hence, the limited life expectancy associated with CF. Recognition of the colonizing or infecting agents of the respiratory tract in individuals with CF may permit specific antimicrobial therapy to be used. However, culture media which permit the isolation of these important bacterial pathogens from complex mixtures of other oropharyngeal flora must be used. Various selective media for *S. aureus*, *H. influenzae*, and *P. aeruginosa* have been described and shown to facilitate detection of these species in sputum (8, 13). Until recently, the recognition of *P. cepacia* in cultures of respiratory specimens has been complicated by the lack of a similarly useful selective culture method for this organism. Gilligan et al. (5) reported a selective medium for isolation of *P. cepacia* from patients, and Wu and Thompson (14) developed a medium intended for isolation of *P. cepacia* from environmental samples. We report the development and evaluation of an additional selective and differential medium and the experience with its use in a multicenter study to examine comparative recovery rates of *P. cepacia* from the respiratory tracts of individuals with CF.

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MATERIALS AND METHODS

Culture media. The medium developed was an oxidation-fermentation (OF) base supplemented with agar, lactose, and two antimicrobial agents. It is referred to as OFPBL. The formulation per liter of medium was 9.4 g of OF basal medium (Difco Laboratories, Detroit, Mich.), 15.0 g of Bacto-Agar (Difco), 10.0 g of lactose (Difco), 300,000 U of polymyxin B sulfate (The Upjohn Co., Kalamazoo, Mich.), and 200 U of bacitracin (Upjohn). The OF base and agar were dissolved in 1,000 ml of distilled water by boiling, autoclaved for 15 min at 15 lb/in^2 (121°C), and cooled to 50°C. The antimicrobial agents and lactose (10 g/100 ml) were sterilized by filtration before being added to the cooled medium. Approximately 20 ml of medium was dispensed into petri plates (15 by 100 mm), allowed to solidify, stored at 4°C, and used within 60 days. The pH of the medium was 6.8 ± 0.1. Other media used for comparison with OFPBL were MacConkey agar (MAC) and xylose lysine-deoxycholate (both prepared according to manufacturer directions; Difco), 5% sheep blood agar (SBA) in Columbia (BBL Microbiology Systems, Cockeysville, Md.) or Trypticase soy agar base (BBL), and 5% Columbia SBA supplemented

with polymyxin B and bacitracin (SBAPB), as described for OFPBL.

Recovery of *P. cepacia* from pure or mixed cultures. Initial tests were performed with stock culture isolates of *P. cepacia* recovered from respiratory specimens of CF patients or from other sites of non-CF patients. Inocula were prepared from 24- to 48-h cultures growing on SBA at 30°C and adjusted to contain ca. 10^3, 10^5, or 10^7 CFU/ml. A bacteriologic loopful of the suspension was streaked over the surface of each medium being tested. Plates were incubated at 30°C and examined for growth of *P. cepacia* at 24, 48, and 72 h. Since the differential property of OFPBL is based on acidification of lactose in the medium, producing a yellow color change, the ability of *P. cepacia* to acidify this medium in the presence of a strong deaminating organism (*Proteus* spp.) was also tested. Suspensions of clinical isolates of *Proteus mirabilis* and *Proteus vulgaris* at 10^7 CFU/ml were mixed with *P. cepacia* at 10^5 and 10^3 CFU/ml and inoculated as described above. To test the recovery of *P. cepacia* among mixed flora and from respiratory tract specimens, a strain of *P. cepacia* was seeded at a density of ca. 10^5 or 10^7 CFU/ml in fresh sputum samples collected randomly from 16 patients with CF. These sputum specimens, simulated to be from patients infected with *P. cepacia*, were cultured on SBA, OFPBL, and MAC, incubated at 30°C, and observed at 24, 48, and 72 h. Isolates were identified primarily by conventional methods (4) and with the aid of supplementary commercial (API 20E, Analytab Products, Plainview, N.Y.; Uni-N/F-Tec, Flow Laboratories, Inc., McLean, Va.; and Autobac, General Diagnostics, Div. Warner-Lambert Co., Morris Plains, N.J.) identification systems. Characteristics used for identification of *P. cepacia* by the conventional methods (4) were oxidase production (+); nitrate reduction (+/−) and nitrogen gas production (−); indole (−), lysine decarboxylase (+), and arginine dihydrolase (−); acidification of oxidative low-peptone medium containing glucose, lactose, maltose, mannitol, sucrose, or xylose; *o*-nitrophenyl-β-D-galactopyranosidase production (+); polymyxin B resistance; and flagellar arrangement (≥2 polar).

Multicenter clinical study. A comparative field trial of the OFPBL medium was designed to collect data from seven university-affiliated hospitals that conducted CF clinics: Children's Memorial Hospital and University of Oklahoma Health Sciences Center, Oklahoma City; Columbus Children's Hospital, Columbus, Ohio; University of Calgary Health Sciences Center, Calgary, Alberta, Canada; North Carolina Memorial Hospital, Chapel Hill; University of Utah Medical Center, Salt Lake City; University of South Alabama Medical Center, Mobile; and Louisiana State University Medical Center, Shreveport. The clinical microbiology laboratories at each center added OFPBL to their routine batteries of media for culturing respiratory specimens from CF patients. OFPBL was furnished to each of the centers by Remel, Lenexa, Kans. At six of the seven centers, specimens submitted consecutively over a 4- to 6-month period were included; specimens for the study were included randomly at one center over a 6-month period. Culture results on OFPBL were compared with results on MAC and SBA for recovery of *P. cepacia*. Each laboratory performed semiquantitative cultures and, in the cases involving sputum specimens, with freshly expectorated, untreated samples. Throat swabs were also acceptable for inclusion in the study. OFPBL plates were incubated at 30°C except at one center, where they were incubated at 35°C, for 5 days. OFPBL plates were examined independently by a worker unaware of the results on the other culture media. Isolates that resembled *P. cepacia* (yellow colonies due to acid production and green-to-yellow change of surrounding medium) were sent to the Oklahoma center for identification. A few isolates were further referred to Robert Weaver, Centers for Disease Control, Atlanta, Ga., for identification. Each center identified *P. cepacia* recovered from MAC and SBA by using its usual methods. Standardized disk diffusion susceptibility testing was performed on all *P. cepacia* isolates.

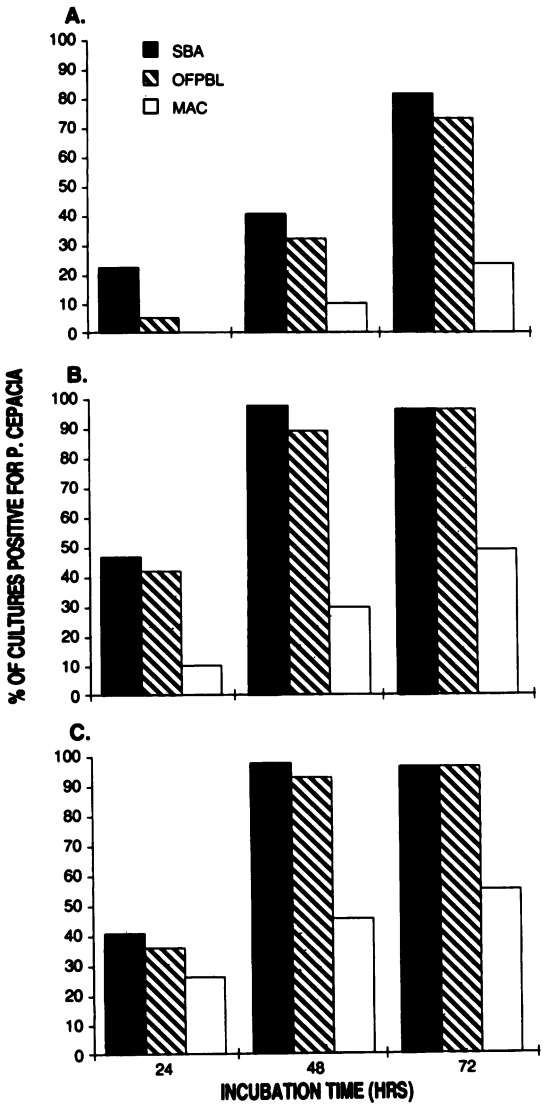

FIG. 1. Support of growth by various media of 22 *P. cepacia* isolates. Inoculum sizes: 10^3 CFU/ml (A), 10^5 CFU/ml (B), and 10^7 CFU/ml (C).

RESULTS

The initial comparison of various media with seven *P. cepacia* strains showed adequate support of growth by SBA, SBAPB, and OFPBL. Poorer growth was observed on MAC than on SBA or OFPBL, and because of poorest support of growth for these strains, xylose lysine-deoxycholate was excluded from further studies. The growth of a larger number of strains (n = 22) was also best supported, at a low or high density of inoculum, by SBA or OFPBL (Fig. 1).

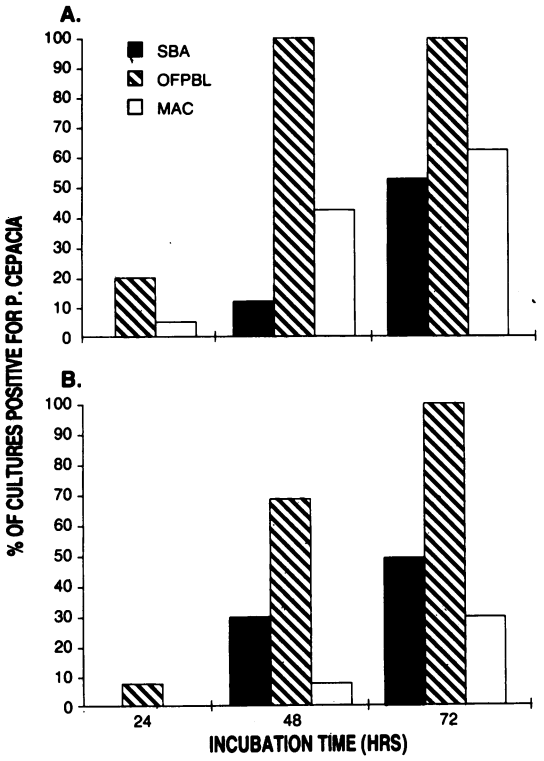

FIG. 2. Recovery of *P. cepacia* from seeded specimens (n = 16) of sputum. Inoculum sizes: 10^7 CFU/ml (A) and 10^5 CFU/ml (B).

Cumulatively, 93% of *P. cepacia* isolates produced a yellow color change in the medium by 72 h, and all were yellow by 5 days. The yellow change caused by *P. cepacia* was due to acid production rather than pigment. Strong alkalinization of the medium by growth of *Proteus* sp. did not mask the color change but delayed it by 24 h. In contrast to these results based on pure cultures, the recovery of *P. cepacia* from simulated specimens was markedly better on OFPBL than it was on either SBA or MAC (Fig. 2). At the lower inoculum, all isolates were recovered on OFPBL incubated for 72 h, and at the higher inoculum all were recovered on OFPBL within 48 h.

Of 725 respiratory specimens from CF patients included in the multicenter study, 58 (8%) yielded *P. cepacia* on OFPBL, 19 (2.6%) yielded *P. cepacia* on MAC ($P < 0.001$, χ^2 test), and 17 (2.3%) yielded *P. cepacia* on SBA. The distribution of specimens and isolates among the various centers is shown in Table 1. The 725 specimens were collected from 428 patients. The overall incidence of *P. cepacia* was 6.1% (26 of 428) and ranged from 14% at Louisiana State University to 0% at the University of Oklahoma (Table 2). The time required to recognize the 58 isolates of *P. cepacia* is shown in Fig. 3. Most were detected by 48 h of incubation, but 3 to 4 days of incubation was required for detection of approximately one-third of the isolates. The yield of isolates from MAC or SBA did not increase after day 3 of incubation.

TABLE 1. Recovery of *P. cepacia* at various centers

Center[a]	No. of specimens	No. (%) positive for *P. cepacia* on:		
		OFPBL	MAC	SBA
CCH	342	23 (6.7)	7 (1.8)	5 (1.2)
UC	107	25 (15)	5 (4.7)	5 (4.7)
OU	96	0	0	0
UNC	85	2 (2.4)	0	0
UU	49	5 (10)	5 (10)	5 (10)
USA	32	1 (3.1)	1 (3.1)	1 (3.1)
LSU	14	2 (14)	1 (7)	1 (7)
Total	725	58 (8)	19 (2.6)	17 (2.3)

[a] CCH, Columbus Children's Hospital; UC, University of Calgary Health Sciences Center; OU, Children's Memorial Hospital and University of Oklahoma Health Sciences Center; UNC, North Carolina Memorial Hospital; UU, University of Utah Medical Center; USA, University of South Alabama Medical Center; LSU, Louisiana State University Medical Center.

TABLE 2. Patient isolates of *P. cepacia*

Center[a]	No. of patients	No. (%) of patients with *P. cepacia*
CCH	196	11 (5.6)
UC	53	6 (11)
OU	49	0
UNC	48	2 (4.2)
UU	38	4 (11)
USA	30	1 (3.3)
LSU	14	2 (14)
Total	428	26 (6.1)

[a] CCH, Columbus Children's Hospital; UC, University of Calgary Health Sciences Center; OU, Children's Memorial Hospital and University of Oklahoma Health Sciences Center; UNC, North Carolina Memorial Hospital; UU, University of Utah Medical Center; USA, University of South Alabama Medical Center; LSU, Louisiana State University Medical Center.

Most *P. cepacia* isolates were recovered as the predominant isolate, and overall, 23% of the specimens yielded a significant amount (>2+, defined by growth of >10 colonies in quadrant 2 of the streak plate) of non-*P. cepacia* growth on OFPBL. The other organisms recovered on OFPBL are shown in Table 3. Although susceptible to polymyxin B by standard testing, *P. aeruginosa* grew on OFPBL at a high inoculum. Several isolates of a species that closely resembled *P. cepacia*, tentatively identified as *Pseudomonas gladioli*, were encountered at three of the centers (University of Oklahoma, Columbus Children's Hospital, and Louisiana State University). Although *P. gladioli* did not produce acid from lactose, all isolates were yellow on OFPBL. The biochemical characteristics and susceptibilities to selected antimicrobial agents of these and the *P. cepacia* isolates are shown in Tables 4 and 5.

DISCUSSION

P. cepacia has been implicated as an important pathogen among CF patients at several centers. Increased rates of

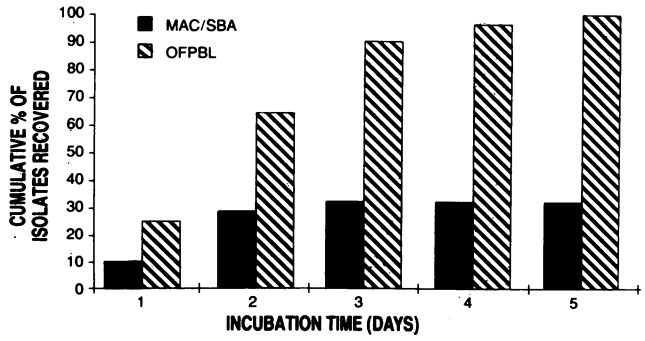

FIG. 3. Duration of incubation required for recognition of *P. cepacia* (58 isolates) from the respiratory tracts of 26 CF patients. MAC/SBA, Either MAC or SBA.

TABLE 3. Organisms other than *P. cepacia* recovered on OFPBL

Organism	No. of specimens
Pseudomonas aeruginosa	111[a]
Pseudomonas maltophilia	2
Pseudomonas fluorescens	1
Pseudomonas stutzeri	1
Pseudomonas gladioli	10
Achromobacter spp.	10
Flavobacterium spp.	1
Acinetobacter spp.	1
Serratia spp.	3
Proteus spp.	2
Yeasts	31
Aspergillus spp.	6
Mycobacterium chelonae (*M. chelonei*)	1

[a] Of 111 specimens, 21 yielded >3+ growth of *P. aeruginosa*.

colonization and deaths associated with *P. cepacia* have been observed over the past 10 to 15 years (7, 11, 12). Patients with *P. cepacia* tend to have a poor clinical status and their antimicrobial therapy is complicated, accentuating the need to detect this organism when it is present. The results of the multicenter clinical study showed that OFPBL medium is superior to MAC or SBA for the isolation of *P. cepacia* from respiratory specimens of CF patients. Our findings suggest a threefold increase in the isolation rate of *P. cepacia* with OFPBL over that with MAC or SBA: *P. cepacia* was recovered from 58 specimens by OFPBL but from only 19 specimens by MAC or SBA. Gilligan et al. (5) found 35 and 21 isolates on PC medium and MAC, respectively, in a similar clinical study between two centers. Tablan and co-workers (10) recently tested the proficiency of microbiology laboratories at CF centers for isolating *P. cepacia* from simulated sputum specimens. Laboratories using the OFPBL as described in our report or PC medium were more likely to isolate *P. cepacia* than were those which did not (95 versus 22%). One of nine centers using PC medium compared with none of five centers using OFPBL failed to isolate *P. cepacia*.

It appears that the improved recovery of *P. cepacia* with OFPBL was primarily due to the selectivity of the medium.

TABLE 4. Biochemical characteristics of patient isolates recovered on OFPBL

Characteristic	% of isolates positive	
	P. cepacia (*n* = 26)	*P. cepacia*-like (*P. gladioli* [*n* = 10])
Growth on MAC	79	100
Indophenol oxidase	93	60
Lysine decarboxylase	79[a]	0
Arginine dihydrolase	0	0
Nitrite production/N_2 gas	41/0	50/0
OF sugars		
Glucose	100	100
Lactose	100	0
Maltose	100	0
Mannitol	100	100
Sucrose	45	0
Xylose	97	100
ONPG[b]	100	70

[a] All lysine decarboxylase-negative isolates were from one center (University of Utah Medical Center).

[b] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.

TABLE 5. Antimicrobial susceptibilities of patient isolates from OFPBL

Antimicrobial agent	% Susceptible	
	P. cepacia (*n* = 26)	*P. cepacia*-like (*P. gladioli* [*n* = 10])
Polymyxin B	0	0
Ticarcillin	17	100
Gentamicin	14	100
Kanamycin	34	90
Trimethoprim-sulfamethoxazole	48	70
Chloramphenicol	17	10

Polymyxin B suppressed gram-negative flora, and bacitracin suppressed the gram-positive and *Neisseria* flora of the respiratory tract. However, despite a concentration of 300 U of polymyxin B per ml of medium, *P. aeruginosa* was recovered frequently on OFPBL. The protective effect of the slime in sputum of CF patients, along with an inoculum effect noted by testing pure cultures of *P. aeruginosa* for growth on OFPBL, possibly explains this observation. Only 2 of 106 clinical isolates of *P. aeruginosa* were encountered with PC medium (5). Although OFPBL may permit growth of *P. aeruginosa*, interference in the recovery of *P. cepacia* was rarely encountered, and colonies of *P. cepacia* were easily recognized by a yellow color change in the medium. Moreover, results of susceptibility testing showed that 17% of the *P. cepacia* isolates were susceptible to ticarcillin at concentrations below the amount in PC medium. The significance of finding *P. gladioli*, formerly known as *Pseudomonas marginata* (3), in association with CF is uncertain. Further clinical and microbiologic study of those cases seems warranted.

Differentiation of flora on OFPBL was most easily accomplished when nonutilizers of lactose, such as *P. aeruginosa*, grew along with *P. cepacia*. *P. cepacia* and other nonfermenters could not be distinguished from fermenters, such as lactose-positive *Serratia* spp., on the basis of acid production from lactose. Therefore, the initial step in further characterization of isolates should include oxidase testing and determination of reactions on a medium such as triple sugar iron agar. Subculture to blood agar before performing the oxidase test was necessary to avoid false-negative oxidase results for *P. cepacia*.

The facilitated recognition of *P. cepacia* in patients with CF should lead to better understanding of the epidemiology and pathogenesis of pulmonary disease attributed to this organism. It is of interest that the isolates of *P. cepacia* from different patients at one center (University of Utah Medical Center) were all of the same, unusual (lysine decarboxylase-negative) biotype. Observations such as this may permit epidemiologic investigations into the source of *P. cepacia* in selected populations to be undertaken.

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